

Scientific Summary of the 3rd International Symposium on Propionibacteria and Bifidobacteria: Dairy and Probiotic applications

The *Propiobifido2010* Symposium dealt with the current advances in dairy and probiotic applications of *Bifidobacterium* and *Propionibacterium* genera. Both Gram-positive bacteria are grouped within the *Actinobacteria* phylum and share some common physiological properties. Research into propionibacteria has been classically oriented to their technological properties in dairy products, particularly Swiss-type cheeses, whilst research on bifidobacteria has mainly been focused on their probiotic potential. Nowadays both, the probiotic potential and the improvement of technological characteristics of propionibacteria and bifidobacteria, are scientific areas of increasing interest. Communications presented in this Symposium focused on five major subjects which are commented on below.

1. Physiology and Metabolism

The physiology and metabolism of propionibacteria and bifidobacteria determine their relationship with the external environment and are the basis of their probiotic properties. They are important determinants of the interaction of propionibacteria and bifidobacteria with other microorganisms from the intestinal environment. In this Symposium it was highlighted that the enhancement of butyrate and gas production after consumption of these bacteria, or in the presence of oligofructose, could be attributed to mechanisms of cross-feeding between bifidobacteria and butyrate-producing intestinal bacteria. Proteins, sugars and other structures from the bacterial cell-envelope participate in the cross-talk with the host or in the interaction with the external environment, then influencing health-promoting or technological properties

of bifidobacteria and propionibacteria. In the Symposium studies on exocellular polysaccharides, lipoteichoic acids, and ABC-type multidrug efflux systems conferring antibiotic resistance in bifidobacteria, as well as bacteriocins and lipolytic esterases from *Propionibacterium freudenreichi* were presented. In addition to the interactions with other intestinal microorganisms and the host, the bacteria present in the intestine will also interact with components of the diet. It was reported in this Symposium that some *Bifidobacterium* strains can convert *in vitro* inactive glycosides of anthocyanins and isoflavones into their corresponding aglycones, probably through the beta-glucosidase microbial activity; aglycones can then undergo chemical or enzymatic degradation towards more biologically active forms. Also reported was the phytase activity of several bifidobacteria strains able to convert anti-nutrient phytic acid, the most abundant form of phosphate storage in vegetables, into myo-inositol triphosphate, some of whose isomers could display health-promoting activities. It has also been stated that milk proteins conjugated to galactose formed via Maillard reaction, as well as lactulose-derived galactooligosaccharides obtained by enzymatic transglycosilation of lactulose, could act as prebiotic substrates.

So far a very challenging issue has been to determine the physiological status of microbes. Traditionally the only distinction that was made was between cultured / alive and un-cultured / dead cells; however, now we know that other statuses do exist. Fluorescent probes allow a more reliable determination of the viability and physiological status of microbial cells than traditional plate counts. Two works were presented on the use of such probes to determine the viability of *Propionibacterium* and some lactic acid bacteria.

2. Industrial and Technological Applications

51 The commercial use of these two microbial groups constitutes the main driving
52 force in this area of knowledge. Some communications addressed the selection and
53 characterization of probiotic properties of bifidobacteria and propionibacteria strains
54 isolated from classical foods (raw milk, Emmental cheese, fermented products....) as
55 well as from new, living being environments. This last case is of the isolation and
56 characterization of propionibacteria strains from the stomach of human adults, these
57 microorganisms displaying high resistance to gastrointestinal stressing factors, or
58 those isolated from the intestine of laying hens for their application in poultry. A
59 significant number of communications dealt with the characterization of bifidobacteria
60 population in infant faeces and on the isolation and characterization of bifidobacteria
61 from human milk for the application of these strains in infant formulas. Studies on
62 probiotic and technological properties were also undertaken in classical and newly
63 designed fermented products containing propionibacteria and bifidobacteria. In this
64 way, it is interesting to remark that miniaturized models of dairy products were
65 optimized and presented in this Symposium for monitoring technological properties
66 and for predicting probiotic behaviour of propionibacteria.

67 Bifidobacteria are generally more sensitive than propionibacteria and lactobacilli
68 to environmental factors. Their viability and stability in foods can be improved by
69 tailoring the production conditions, by utilization of protectants and new technologies
70 (i.e. by microencapsulation), or by adaptation to stressing conditions. In general,
71 stress-tolerant mutants became more resistant to technological harsh conditions than
72 their parental strains, thus contributing to their better survival in both food matrices
73 and gastrointestinal tract. In addition, adapted strains represent an alternative
74 strategy to study genetic variants of a strain under stressful conditions when no other
75 genetic tools are easily available. Thus, works on heat-shock and aerobic tolerant

derivative *Bifidobacterium* strains were presented in this Symposium and were characterized by genomic and proteomic approaches.

3. Health Effects: Clinical and *in vivo* Data

The use of probiotics in medicine is steadily increasing as demonstrated by more than 600 records currently contained in the Cochrane Central Register of Controlled Trials testing the efficacy of probiotics for different health applications. Specifically, in the frame of the applications of probiotics in gastroenterology, three human intervention studies were presented in this Symposium. One of them using yoghurt supplemented with a *Bifidobacterium longum* strain for alleviation of constipation in children and adolescents in Brazil. The other two works analyzed intestinal microbiota dynamics of adults fed yoghurt containing commercial bifidobacteria and lactobacilli strains, in Canada and Spain. In the last case, adults were previously submitted to antibiotic treatment. Remarkably, some new and very promising applications tested in animals by the administration of bifidobacteria were presented in this Symposium. They include the amelioration of endotoxemia in diabetes type II and metabolic syndrome and the regulation of the composition of host fat tissue by host-microbe-diet interactions.

It is evident that more human intervention studies are needed in order to accomplish requirements from the European Food Safety Authority (EFSA) for the approval of health claims in probiotic-containing products in Europe. To help in this task, and before accomplishing time-consuming and expensive *in vivo* and human intervention studies, a previous screening of the probiotic potential of candidate strains would be useful and very convenient. Relating to this, in this Symposium an *in*

vitro cellular model was presented to test the efficacy of putative probiotic strains against gut dysfunctions related to Irritable Bowel Syndrome (IBS).

4. Health Effects: Mechanisms

The mechanisms of health promotion by probiotics can include modulation of intestinal microbiota, improvement of the gut barrier, competitive exclusion or displacement of pathogens, and immune modulation. A body of work was presented in this Symposium on the immune-stimulatory effect of *Bifidobacterium* and *Propionibacterium* strains and of some specific sub-cellular components of these microorganisms such as surface polysaccharides or proteins. *In vitro* studies have been directed to test the effect on different cell-components of the human immune system: maturation patterns of dendritic cells and T-helper lymphocytes, stimulation of natural killer cells, B- and T-lymphocytes, and cytokine production patterns by natural killer, dendritic and peripheral blood mononuclear cells. Aggregation capacity and adhesion to intestinal epithelial cell lines has been underlined as important *in vitro* characteristics that could be related with the ability to modulate the immune system by intestinal bacteria. In this regard, some communications presented in this Symposium investigated adhesion to human intestinal cell lines and the aggregating capability of several *Bifidobacterium* strains in an attempt to correlate these properties with their immunomodulating potential. Some *P. freudenreichii* and *Bifidobacterium* strains (*B. longum* and *Bifidobacterium bifidum*) have shown promising immunomodulatory capability and good perspectives for the alleviation of Inflammatory Bowel Disease (IBD) symptoms when tested using *in vivo* models of colitis in mice. A study was also presented assessing the efficiency of a *B. longum* strain against rotavirus infections in a mouse model.

5. “Omics” and Molecular Approaches

Sequencing and functional analysis of genomes from *P. freudenreichii* and several *Bifidobacterium* species presented in this Symposium are significantly contributing to improving our understanding of the adaptation capability of these microorganisms to different environments. In the case of bifidobacteria, different species can differ in their adaptation strategies for the use of the diverse components of diet, also displaying different ways of interaction with other members of the intestinal microbiota. On the other hand, the application of genetic tools, such as microarray technology, knock-out insertional mutation and others, is contributing to the rapid advance of our knowledge of some metabolic pathways and functionality of several proteins of interest. A huge effort has been made in this area in the species *B. breve*; the characterization of a two component regulatory system, a serpin, and some mechanisms of bile stress response and the metabolism of some sugars are under investigation and some results were presented in the Symposium. The availability of genome sequences is facilitating the development of typing techniques, such as multilocus sequence typing, of strains in species of *Propionibacterium* and *Bifidobacterium*.

It is expected that within a short period of time significant advances in our understanding of the functionality and behaviour of some microorganisms of these two bacterial genera will occur. The large increase of immunomodulatory studies presented in *Probiobifido2010*, with regard to the previous Symposia, highlights the interest of the Scientific Community to unravel the mechanisms that direct the cross-talk between proionibacteria and bifidobacteria and the human host. It is also

150 expected that the new beneficial actions that are being demonstrated in animal
151 models, as well as the requirements of the new European Regulation on Health
152 Claims in food products, will force an increase in the number of human intervention
153 studies. *Propiobifido2010* provided the opportunity to present and discuss the latest
154 scientific advances for people interested in propionibacteria and bifidobacteria. For
155 sure, the forthcoming *Propiobifido2013* Symposium will deliver exciting news in this
156 research field.

157

158 **Clara G. de los Reyes-Gavilán**

159 Chair of the Symposium *Propiobifido2010*

160 Instituto de Productos Lácteos de Asturias - Consejo Superior de Investigaciones

161 Científicas (IPLA-CSIC)

162 Carretera de Infiesto s/n, 33300 Villaviciosa, Asturias, Spain

163 greyes_gavilan@ipla.csic.es